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How To Tell a Sea Monster: Molecular Discrimination of Large Marine Animals of the North Atlantic

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"Either we do know all the varieties of beings which people our planet, or we do not. If we do not know them all—if Nature has still secrets in the deeps for us, nothing is more conformable to reason than to admit the existence of fishes, or cetaceans of other kinds, or even of new species . . . which an accident of some sort has brought at long intervals to the upper level of the ocean."

—*Jules Verne,* Twenty Thousand Leagues Under the Sea, *1870*

Abstract. Remains of large marine animals that wash onshore can be difficult to identify due to decomposition and loss of external body parts, and in consequence may be dubbed "sea monsters." DNA that survives in such carcasses can provide a basis of identification. One such creature washed ashore at St. Bernard's, Fortune Bay, Newfoundland, in August 2001. DNA was extracted from the carcass and enzymatically amplified by the polymerase chain reaction (PCR): the mitochondrial NADH2 DNA sequence was identified as that of a sperm whale (*Physeter catodon*). Amplification and sequencing of cryptozoological DNA with "universal" PCR primers with broad specificity to vertebrate taxa and comparison with species in the GenBank taxonomic database is an effective means of discriminating otherwise unidentifiable large marine creatures.

Introduction

At least since the *Iliad*, the possible occurrence of unusually large, exotic marine creatures has exerted a powerful hold on the human imagination. Professor A. C. Oudemans' 1892 book *The Great Sea Serpent* described more than 200 reports of unknown marine creatures (Ley, 1959). Ellis (1994) gives a contemporary list. Even in the first year of a new century when the complete human genome has become known (International Human Genome Sequencing Consortium, 2001), the possibility that entirely new, previously unknown species may unexpectedly present themselves remains tantalizing. Discovery in the last century of the first coelacanth (*Latimeria*), the "megamouth" shark (*Megachasma*) and, most recently, a second species of coelacanth in the waters off Sulawesi in Indonesia (Holder *et al.,* 1999) keeps us alert to the possibility of "new varieties of beings" in the deeps. Modern methods of phylogenetic systematics, based on detailed morphological and molecular analyses, have made it possible to place such discoveries in their evolutionary context.

Morphological analysis of putative new species may be hampered by incomplete or poorly preserved material; in such cases, molecular biology may hold the key to natural history. Enzymatic amplification by the polymerase chain reaction (PCR) (Saiki *et al.,* 1988) of the minute amounts of DNA persisting in ancient or forensic biological material has been shown to be an effective means of individual and species identification (Herrmann and Hummell, 1994). The extra-nuclear mitochondrial (mt) DNA genome has been particularly valuable, as more than a decade of molecular systematic work has provided an extensive database ("GenBank") of molecular "type" sequences for many species of

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Figure 1. "Sea monster" discovered at St. Bernard's, Fortune Bay, Newfoundland, 2 August 2001. The maximum length of the carcass is 5.6 m. The transverse cuts were made during dissection. Note the lobes at the right-hand end on the side facing the camera.

marine sharks, fish, and mammals (Wheeler *et al.,* 2000; Benson *et al.,* 2000). Routine species identification of subfossil material hundreds or thousands of years old (Hofreiter *et al.,* 2001) is possible, as is forensic determination of questioned species in commercial products such as salted or dried fish (S. M. Carr and H. D. Marshall, unpubl. obs.), or processed whale meat (Baker *et al.,* 1996) including that from endangered species (Palumbi and Cipriano, 1998). We report here what appears to be the first successful use of PCR-based recovery of DNA to identify a "sea monster."

On 2 August 2001, residents of the community of St. Bernard's, Fortune Bay, on the south coast of the island of Newfoundland, were confronted with an enormous, whitish mass of rotting flesh that had washed up on a local beach overnight. They contacted the Department of Fisheries and Oceans in St. John's, who sent experts to examine the carcass (Fig. 1). The remains were about 5.6 m long and 5.0 m wide. Neither head nor tail was present: the carcass consisted primarily of bleached tissue. The surface was rough and fringed with material initially characterized as "hair," which upon closer inspection appeared to consist of abraded tissue mixed with seaweed and sand. There were seven or eight lobes or slits that extended roughly one-third the length of one side from one end; the last two slits did not extend to the outer margin. No lobes were present on the opposite side, but tissue had evidently been lost from that side. The remainder of the mass tapered slightly. No soft tissue or bones were present; dissection of the side opposite the lobes revealed a small amount of cartilage. The surface layer retained a structure consistent with muscle, but the interior had decomposed to an amorphous mass. Definite identification was impossible due to the state of decomposition and the absence of any remaining external features. The size and general morphology were consistent with either a large shark, such as a basking shark (*Cetorhinus maximus*), or one of the several species of large cetaceans present in Newfoundland waters. The possibility of a giant squid (*Architeuthis dux*) was excluded due to general morphology (Aldrich, 1991).

Materials and Methods

Scientists from the Department of Fisheries and Oceans removed a number of pieces of tissue from just under the exterior of the carcass. DNA was extracted in duplicate from four pieces of tissue by a protease-based method with a QIAamp® DNA Mini Kit (Qiagen, Inc.), according to the manufacturer's instructions.

On the basis of the availability in GenBank of sequences for the mitochondrial (mt) DNA NADH subunit 2 gene (hereinafter NADH2) for a variety of shark (Naylor *et al.,* 1999) and whale species, we performed a series of polymerase chain reaction (PCR) experiments with two forward and reverse primer pairs that amplify a 1103-bp region that

includes the complete NADH2 gene as two overlapping regions of 564 and 711 bp, respectively:

p8F: 5′ aagctatcgggcccataccc 3′ and
p8R: 5′ tttagtcctcctcagcctcc 3′

p9F: 5′ cataatcctactcacatgac 3′ and
p9R: 5′ cttacttagggctttgaagg 3′

PCR reactions were carried out as a two-step procedure designed to enhance amplification of dilute DNA or of DNA with a poor match between template and primer. In this strategy, an initial set of PCR cycles with dilute primers (2.5% of usual concentration) generates a small quantity of amplified template with ends that have an exact match to the primers. A second phase follows, with primers at standard concentration to produce sufficient template for sequence analysis. In the first stage of the procedure, we prepared 25-μl reactions containing 1X PCR reaction buffer and 1 U of *Taq* polymerase (Roche Molecular Biochemicals, Inc.), 10 n*M* of each primer (Cortec DNA Service Laboratories, Inc.), 100 μM of each deoxynucleoside triphosphate (dNTP; Amersham Pharmacia Biotech), and 2 μl of template DNA. Following an initial incubation at 93 °C for 3 min, samples were taken through 23 cycles, each comprising denaturation at 93 °C for 45 s, annealing at 45 °C for 45 s, a ramp from 45 °C to 55 °C over 45 s, and extension at 72 °C for 1 min. The last cycle was followed by a further extension at 72 °C for 10 min. In the second stage, a second 25-μl reaction volume was added to each reaction tube, containing 1X PCR reaction buffer, 1 U *Taq* polymerase, 800 n*M* each primer (so as to bring the final concentration of each to 400 n*M* in a 50-μl volume), and 100 μM of each dNTP. Samples were taken through an additional 45 cycles of PCR, as described above. All thermal manipulations were achieved using the GeneAmp PCR System 9600 (Perkin-Elmer).

PCR product sizes were verified by electrophoresis of 5 μl of the product through 2% agarose in 1X TBE buffer followed by ethidium bromide staining. Excess primer and nucleotides were removed from the PCR products using a QIAquick® PCR Purification Kit (Qiagen, Inc.).

DNA sequencing was accomplished by fluorescent dye-terminator chemistry carried out on an Applied Biosystems 3700 Automated DNA sequencer (Qiagen Genomics Sequencing Service, Qiagen Genomics Inc.) with the same primers that were used for amplification.

Results

No special difficulties were encountered in DNA extraction. Agarose electrophoresis of small portions of the extracted product indicated the presence of high molecular weight DNA in all but one of the tissue samples. Amplification with the two pairs of NADH2 primers was successful in about one-half of trials, and produced amplification products of the expected size. Inspection of electrophoretic separations indicated clean but weak amplification. Products from three replicate amplifications of each region were pooled for DNA sequencing.

A complete NADH2 sequence was obtained by assembly of the overlapping forward and reverse sequences of the two amplified regions. A BLAST search against the complete GenBank database indicated that the composite sequence had a 99.6% (1040 out of 1044 bp) match with the published NADH2 sequence for a sperm whale (*Physeter catodon*) (Árnason *et al.*, 2000: GenBank accession NC002503). The four nucleotide differences included one second codon position C ↔ T transition difference that would be expected to result in a threonine ↔ methionine amino acid difference between the GenBank type and Fortune Bay sequences, respectively. The magnitude of the differences is consistent with expected intraspecific variation. The DNA sequence was submitted to GenBank and assigned the accession number AF414121.

Discussion and Conclusions

The Fortune Bay "sea monster" is the carcass of a sperm whale (*Physeter catodon*). Sperm whales are the largest of the toothed whales (Odontoceti), they are not uncommon in the waters off the southern shore of the island of Newfoundland (Leatherwood *et al.*, 1976), and strandings of more or less intact whales are not infrequent (G. B. Stenson, unpubl. data). The carcass appears to be a mass of decomposing muscle tissue that has separated from the vertebral column and ribs. The peripheral lobes, which might be mistaken for a set of chondrichthian gill arches, are consistent with intercostal flesh. The feathery or hairy appearance is apparently abraded tissue. Exact postmortem age of the carcass is impossible to determine, but it is likely to have been in the water a long time.

Accounts and pictures in the popular press indicate that carcasses resembling the one found in Fortune Bay have washed up in several oceans of the world; some of these have attracted international media attention. Verrill (1897) initially described a large, whitish carcass that appeared in St. Augustine, Florida, as a new species of giant octopus, though he later withdrew this identification. Original newspaper reports in 1962 of a Tasmanian creature dubbed the "Globster" described it in the following terms: "It was initially covered with fine hair. . . . There were five or six gill-like hairless slits on each side of the fore part. There were four large hanging lobes in the front, and between the center pair was a smooth, gullet-like orifice. The margin of the hind part had cushion like protuberances . . . and each of these carried a single row of spines, sharp, and hard, about as thick as a pencil and quill-like. . . [It had] a resilient flesh which appeared to be composed of numerous tendon-like threads welded together in a fatty substance. . . ." (quoted in Ellis, 1994). There was no bone. A later scientific investigation reported the carcass as 8 feet long, 3 feet wide, 10

Table 1

Large marine animals of the North Atlantic

Name and taxonomy	Max. size (meters)	GenBank accession	
		NADH2	Cyt *b*
Elasmobranchii (sharks)[1]			
thresher shark (*Alopias vulpinus*)	6	U91432	U91442
white shark (*Carcharodon carcharias*)	8	U91426	L08031
basking shark (*Cetorhinus maximus*)	13	U91429	U91439
whale shark (*Rhincodon typus*)[2]	18	—	—
Greenland shark (*Somniosus microcephalus*)	7	—	—
Mysticeti (baleen whales)[3]			
bowhead whale (*Balaena mysticetus*)	20	—	X75588
Minke whale (*Balaenoptera acutorostrata*)	10	X87775	X75753
Sei whale (*Balaenoptera borealis*)	19	—	X75582
blue whale (*Balaenoptera musculus*)	26	NC001601[5]	
finback whale (*Balaenoptera physalus*)	27	NC001321[5]	
beluga (*Delphinapterus leucas*)	6	—	X92531
northern right whale (*Eubalaena glacialis*)	16	—	—
humpback whale (*Megaptera novaeangliae*)	16	—	X75584
Odontoceti (toothed whales)[3]			
Atlantic pilot whale (*Globicephala melaena*)	6	—	AF084056
killer whale (*Orcinus orca*)	9	—	X92532
sperm whale (*Physeter catodon*)	21	NC002503[5]	
Cephalopoda (squids & relatives)			
giant squid (*Architeuthis dux*)[4]	18	—	—

[1] Size data from Scott and Scott (1988).
[2] Not known to occur in Canadian Atlantic waters.
[3] Size data from Leatherwood *et al.* (1976).
[4] Aldrich (1991).
[5] Both sequences contained in the accession for the complete mtDNA genome.

inches thick, and without spines. A 1988 report from Bermuda described a "Glob," "2 1/2 to 3 feet thick . . . very white and fibrous with five 'arms or legs,' rather like a disfigured star. . . . It had no bones, cartilage, visible openings, or odor. . . ." (quoted in Ellis, 1994). In 1990, another carcass washed ashore in the Hebrides Islands, Scotland: "It had what appeared to be a head at one end, a curved back and seemed to be covered with eaten-away flesh or even a furry skin and was 12 feet long [and] it had all these shapes like fins along its back. . . ." (L. Phitts to S. McLean, Hancock Museum, Newcastle, UK; pers. comm. to S. M. Carr). Definitive species identification of any of these carcasses has been impossible. Pierce *et al.* (1995) concluded on the basis of ultrastructure and amino acid analysis that the Bermuda carcass was the remains of a vertebrate, and that Verrill's "giant octopus" was actually whale blubber.

In common with the Fortune Bay carcass, these and other reports (see Ellis, 1994) describe oblong whitish carcasses, several meters in length, bordered at one end with fleshy lobes, fringed with feathery white material resembling hair, and without apparent bone or cartilage. In contrast with other reports, we did not find the surface especially difficult to cut, and close examination revealed the presence of cartilage. We suggest that these and other similar remains are likely of cetacean origin. Given the variation in size of the various carcasses, it would be of interest to know the species diversity among such remains. Dead basking sharks present a somewhat different appearance. Decomposition of beached carcasses is accompanied by erosion of the caudal fin and sloughing of the head, leaving a long bony "tail" and a small chondrocranium "head" (Scott and Scott, 1988). A 1977 discovery in the nets of a Japanese fishing trawler was initially described as a "plesiosaur," but detailed morphological examination strongly suggests chondrichthian origins (Kuban, 1997). In future, discrimination of such carcasses should be possible by the means described here.

Because mtDNA is present in high copy number (~1% of total DNA) in vertebrate cells, any particular mitochondrial gene is far more likely to survive postmortem degradation, enzymatic breakdown, and mechanical damage than is any typical single-copy nuclear sequence (Hermann and Hummel, 1994). Here, adventitious "pickling" by prolonged immersion in cold seawater has left enough intact DNA for positive identification. Hofreiter *et al.* (2001) caution that care must be taken to avoid contaminating the minute amounts of DNA present in forensic material with exogenous species: in this case, no (other) sperm whale tissue was present in the laboratory, and appropriate experimental controls were always negative.

When anatomical identification is not possible and DNA can be recovered, effective identification of unknown marine creatures begins with PCR amplification with "universal" primers designed to be homologous to gene regions that are evolutionarily conserved across a diversity of taxa (Kocher *et al.,* 1989; Palumbi, 1996). The resultant DNA sequence can then be compared against the complete GenBank database of the National Center for Biotechnology Information (NCBI) [http://www.ncbi.nlm.nih.gov] by means of a BLAST search (Altschul *et al.,* 1997). This involves a simple "cut and paste" submission of the sequence data over the Internet: an answer is usually obtained within minutes (here, in under 30 seconds). The search returns a set of matches, ranked in order of degree of sequence similarity. In this case, an essentially exact match was obtained, which indicates a positive species identification.

Were a more inexact match to be obtained, phylogenetic analysis would be necessary to ascertain or at least narrow species affinities among the usual suspects. The GenBank taxonomy database currently comprises DNA sequences from more than 50,000 species, of which more than 9600 are vertebrate species, including 110 Elasmobranchii and 80 Cetacea. Reference sequences for the mitochondrial NADH2 gene, the cytochrome *b* gene, or both are available from 3 of the 4 species of sharks and 10 of the 11 species of whales that are found in Atlantic Canadian waters and exceed 6 m in length (Table 1). Failure to obtain positive identification through GenBank does not necessarily indicate an unknown species, but may instead indicate a previously recognized species for which genetic data, or data from a particular locus, are as yet unknown. Continuing studies in marine biology and molecular systematics will improve the range and depth of our knowledge of the genetics of these species, and should provide exact tests for future cryptozoological specimens.

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